DNA looping by FokI: the impact of twisting and bending rigidity on protein-induced looping dynamics

Authors

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The authors wish it to be known that, in their opinion, the first three authors should be regarded as joint first authors.

Supplementary Figure 1

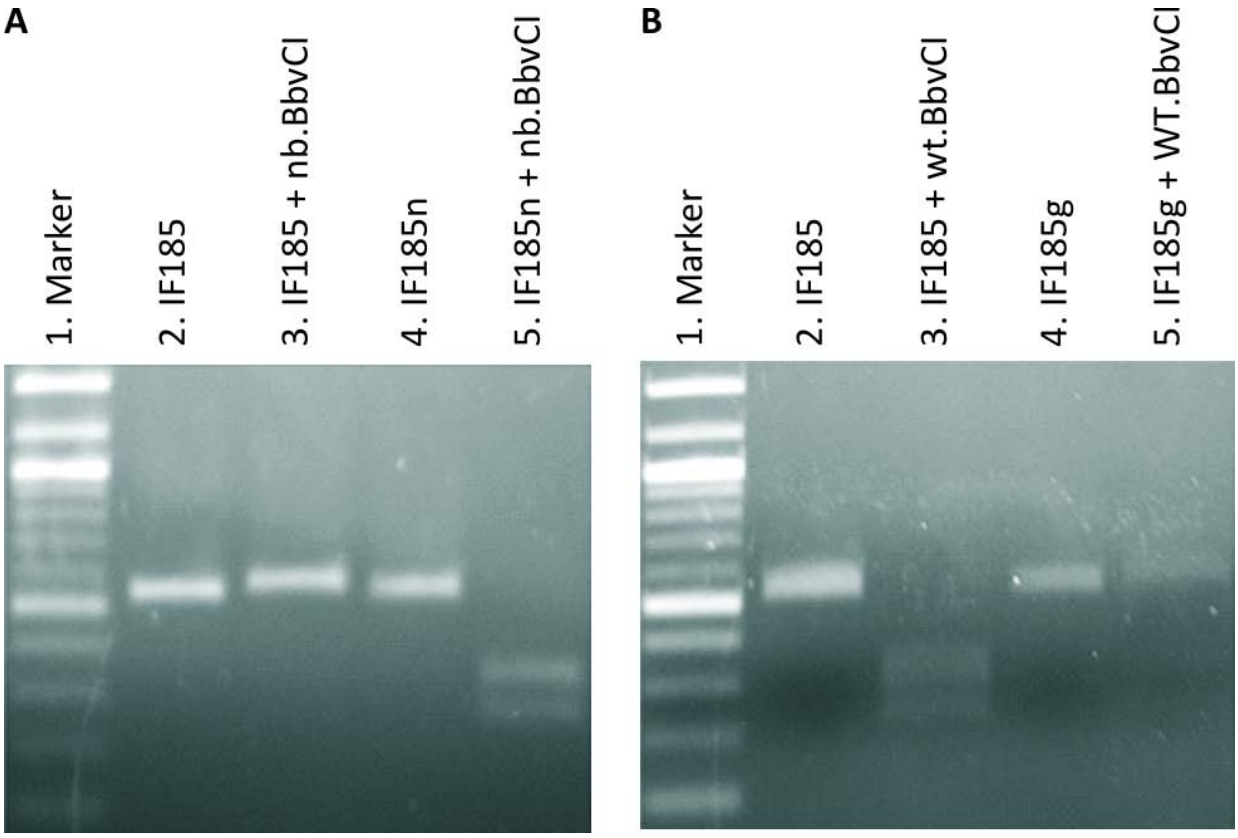

Figure S1. Introduction of a single-stranded nick or a 4-nt gap between FokI recognition sites. Experiments with the IF substrate containing 185 bp are shown as an example. (A) Nicking analysis. Samples of the purified PCR product (IF185) and the nicked construct after digestion with Nt.BbvCI ($IF185_n$), both before (lanes 2 and 4) and after (lanes 3 and 5) reactions with Nb.BbvCI, were subjected to electrophoresis through agarose. The gel shows that the Nb.BbvCI reaction on the $IF185_n$ DNA resulted in a double-strand break at the BbvCI site, while it gave no double-strand breaks on the intact IF185 construct. (B) Gapping analysis. Samples of the purified PCR product (IF185) and the gapped construct that had been made with Nt.BbvCI and Nt.BsmAI ($IF185_g$), both before (lanes 2 and 4) and after (lanes 3 and 5) digestion with native BbvCI, were subjected to electrophoresis through agarose. The intact DNA (IF185) was cut into two fragments by BbvCI while the gapped construct ($IF185_g$) was not cut by BbvCI: the gap removes part of the BbvCI recognition site. In both gels, lane 1 corresponds to a set of 100 bp markers, with a bright band at 500 bp.

Supplementary Figure 2

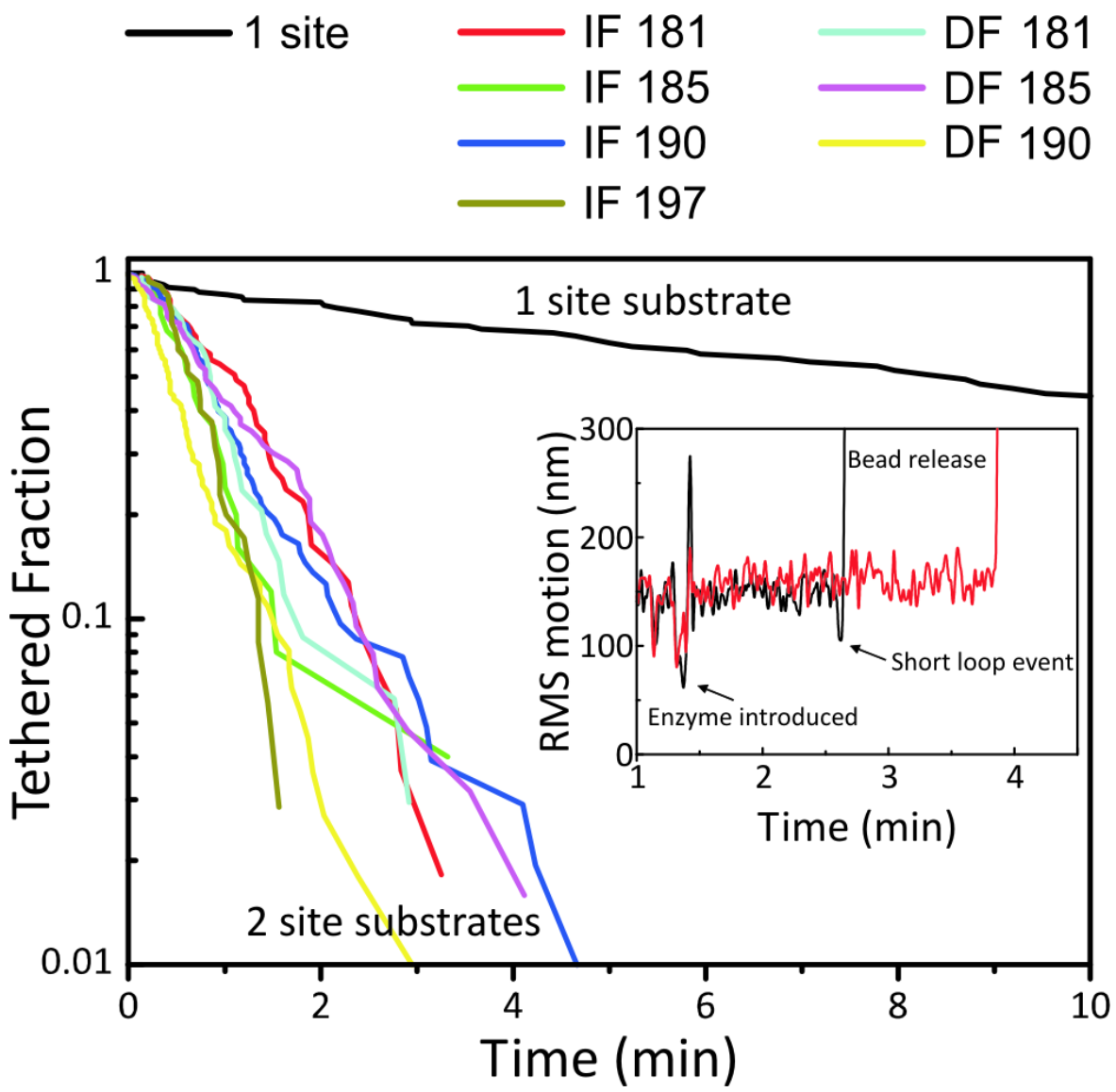

Figure S2. TPM experiments in Mg^{2+}. To demonstrate that FokI is active in our TPM assay, the rate of bead release (plotted as tethered fraction versus time) was determined for constructs containing either one or two FokI recognition sites. The DNA with two FokI sites are in either IF (inverted) or DF (directly-repeated) configurations, with inter-site separations (181 – 197 bp) as indicated above the graph: 1-site denotes the equivalent PCR product from pF1, the plasmid with one FokI site. Rates were determined by flowing 55.6 nM FokI into the observation chamber while monitoring the RMS motion of the tethers. When the enzyme is introduced in the flow cell, the tethers are stretched in the flow and therefore the RMS motion is affected. The sharp rise in the RMS trace indicates the moment the bead is liberated from the tether: i.e., when the protein lets go of the ends of the DNA after it has been cleaved (insert). Half times for bead release fall in the range ~ 30 to 60 s for the two-site constructs but are an order of magnitude slower for the tether containing only one FokI site (800 ± 20 s). Hence, in the TPM set-up, DNA hydrolysis on the two site substrates occurs via loop formation as in free solution.

Supplementary Figure 3

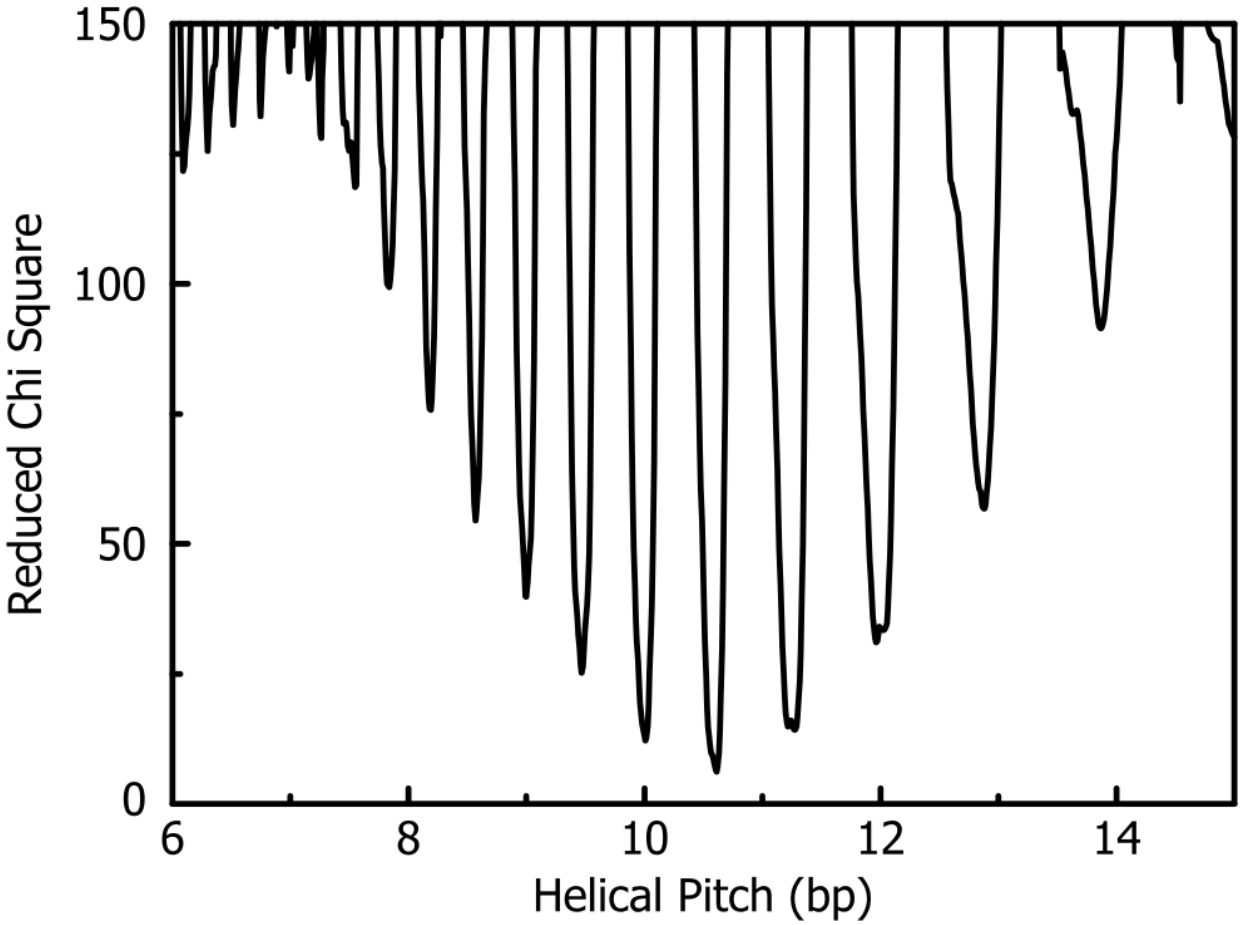

Figure S3. Helical peridodicity. The rates for loop capture, and for loop release (from both negative and from positivetorque) were measured on the complete set of IF DNA constructs. As these constructs maintain essentially the same sequence between the FokI sites, they should all have the same helical repeat. Therefore, all three data sets were fitted to an Arrhenius law (Figure 4, main text) using a single value of the helical pitch as a global variable. The entire reduced chi square landscape for all variables was mapped to find the global minimum. Shown here is the projection along the helical pitch axis, from which the global minimum corresponds to the best fit to the data: it results in a pitch of 10.6 ± 0.1 bp per helical turn. The error was established by finding the values that lies directly to the left and right of the minimum at twice the reduced chi squared value.